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High-voltage-activated Ca^{2+} currents and the excitability of pyramidal neurons in the hippocampal CA3 subfield in rats depend on corticosterone and time of day

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Abstract

This study tested the time-of-day dependence of the intrinsic postsynaptic properties of hippocampal CA3 pyramidal neurons. High-voltage-activated Ca^{2+} currents and the Ca^{2+}- and voltage-dependent afterhyperpolarizations were examined in slices of rat brains obtained at four distinct time periods. Just after onset of the dark phase, the steady-state amplitude of the Ca^{2+} current (-1.24 ± 0.11 nA) was significantly greater ($P < 0.03$) than that of the light phase (-0.84 ± 0.06 nA). Over the entire time range, the amplitude of the Ca^{2+} current correlated with plasma corticosterone levels in a U-shaped function. Furthermore, depolarization-induced excitability during the dark phase exhibited an increased spike after depolarization (3.1 ± 0.1 mV) and a slower adaptation of the firing frequency ($146 \pm 18\%$). These findings point to a dynamic time-of-day dependence of the CA3 neuronal properties and postsynaptic Ca^{2+} currents.

Keywords: CA3; Circadian rhythm; Ca^{2+} currents; Afterhyperpolarization; After depolarization; Corticosterone

Within the brain, various central processes such as extracellular transmitter concentrations or receptor expression are generated as diurnal functions [4,11,15]. Some of these fluctuations occur independently of light cues, the most important 'zeitgeber' [4]. Diurnal rhythms have also been reported for the strength of synaptic connections within the dentate gyrus and the induction of long-term potentiation (LTP) in the CA1 region of the hippocampal formation [2,7,14], which even persist in in vitro preparations [14]. We hypothesized that the daytime-dependent modulation of hippocampal neurotransmission may depend on changes in the influx of Ca^{2+}, which is an important element of hippocampal synaptic plasticity, of postsynaptic excitability, and of neurotransmitter release [9,10,16,17]. This hypothesis was examined by measuring high-voltage-activated (HVA) Ca^{2+} currents of CA3 pyramidal neurons in hippocampal slices from rat brains, isolated at several distinct phases of the light-dark (LD) cycle. In addition, postsynaptic excitability for resting properties and the (partially) Ca^{2+}-dependent afterhyperpolarizations (AHPs) were examined. Since the adrenocortical hormone corticosterone is released diurnally and interacts with central nervous structures such as the hippocampus [8], we investigated the corticosterone-induced modulation of CA3 neuronal excitability.

Adult male Wistar rats (Winkelmann, Borchen, Germany) were group-housed, with food and water ad libitum. The LD cycle was set at 12L:12D with lights on at 06:00 h, referred to as circadian time zero (CT 0). At four distinct time-points, CT 4 and CT 8 (lights on), CT 13 and CT 23 (lights off), rats were decapitated, their brains rapidly removed, and transverse hippocampal slices (400 μm) cut in ice-cold oxygenated (95% O_2 / 5% CO_2) artificial cerebrospinal fluid (ACSF, in mM: 125 NaCl, 2.5 KCl, 1.25 Na_2HPO_4, 4 $MgSO_4$, 26 $NaHCO_3$, 1.3 $CaCl_2$, 1 L(+)-ascorbic acid, 14 D(+)-glucose, all from Merck, Darmstadt, Germany). Trunk blood was collected in ethylenediaminetetraacetate tubes, and centrifuged, and plasma samples were analyzed for free corticosterone using a scintillation proximity radioimmunoassay (Amersham Pharmacia Biotech). All animal experiments were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/EC).

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Hippocampal slices were transferred to a recording chamber perfused with warmed oxygenated ACSF (32°C), and neurons were visually selected for patch-clamp recording. Borosilicate pipettes with a final resistance of 2–4 MΩ were connected to an Axopatch 200B amplifier (Axon Instruments, Foster City, CA, USA), and data were collected with PULSE software (HEKA, Lambrecht, Germany). Current-clamp was performed with an intracellular solution in the pipette (in mM): 120 $KMeSO_4$ (ICN, Eschwege, Germany), 2 $MgCl_2$, 20 KCl, 4 NaCl, 10 HEPES, 0.1 EGTA, 3 Na-ATP, 14 phosphocreatine, and 0.3 Na-GTP. The extracellular solution contained ACSF with 2.5 mM $CaCl_2$, 3.5 mM KCl, 20 μM 6-cyano-7-nitroquinoxaline-2,3(1H,4H)-dione (CNQX), and 10 μM bicuculline (Tocris, Bristol, UK). Isolation of Ca^{2+} currents was performed with an intracellular solution composed of (in mM): 115 $CsMeSO_4$, 3 $MgCl_2$, 10 EGTA, 10 HEPES, 20 TEA-Cl, 4 Na-ATP, 0.3 Na-GTP, 14 phosphocreatine, and 50 units/ml creatine-phosphokinase (all from Sigma Aldrich). The pH was adjusted to 7.2 with CsOH (Fluka, Buchs, Germany) and the osmolarity set to 290 mOsm. The extracellular medium contained (in mM): 95 NaCl, 2.5 KCl, 2 $MgCl_2$, 26 $NaHCO_3$, 1.5 $CaCl_2$, 1 L(+)-ascorbic acid, 14 D(+)-glucose, 20 TEA-Cl, 5 4-aminopyridine (Sigma Aldrich), 5 CsCl, and 0.005 Tetrodotoxin citrate (Tocris). In voltage-clamp mode, series resistance values amounted to 7 MΩ, maximally 40% compensated. While Ca^{2+} currents peaked with ~3 nA and late currents were 1.5 nA, the uncompensated steady-state voltage-control was determined at 13 mV for peak currents and ~6 mV for late currents. A P/8 leak protocol compensated the leak currents and the remaining slow capacitance transients.

A total of 44 CA3 pyramidal neurons were included for analysis of Ca^{2+} currents. Neurons were kept at a −50 mV holding potential which predominantly inactivates low-voltage-activated (LVA) Ca^{2+} currents [1], and therefore isolates HVA Ca^{2+} currents (Fig. 1A). This was tested further by the application of the non-selective HVA Ca^{2+}-blocker, Cd^{2+} [1]. Twenty micromolar Cd^{2+} blocked 45 ± 5% ($n = 2$) of the late current, and 100 μM Cd^{2+} blocked 87 ± 8% ($n = 5$) of the late current, indicating that a large fraction is carried by HVA currents. Comparison of the amplitudes of the *I-V* relation of late Ca^{2+} currents revealed circadian time as a predominant effect for step potentials of −30, −25, −20, −10 mV and 0 mV ($P < 0.05$ for all). Amplitudes were subsequently averaged between −30 and −10 mV, and plotted against CT (Fig. 1B). One hour after lights off (CT 13), Ca^{2+} currents were larger than during the lights-on phases CT 4 (−1.24 ± 0.11 nA vs. −0.82 ± 0.13 nA, Tukey HSD post-hoc $P < 0.025$) and CT 8 (−0.84 ± 0.06 nA, $P < 0.031$). There was no change detected over time during the storage of the slices (max. 10 h), suggesting that there is no CT modulation of Ca^{2+} currents under in situ conditions. Monoexponential fits of inactivation time courses between the initial and late amplitude of the currents at −10 mV yielded comparable

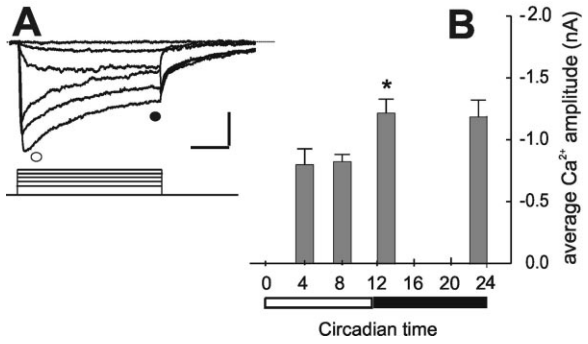

Fig. 1. (A) Typical traces of CA3 pyramidal whole-cell HVA Ca^{2+} currents. The inward currents elicited at −50, −35, −25, −15, −5, and +5 mV are shown. The late phase of the inward current amplitude (●) was measured for each cell. Scale bar: 1 nA, 200 ms. (B) The average amplitudes between −30 and −10 mV are plotted against circadian time (CT). Current amplitudes exhibited a significant time-dependence (ANOVA, $F[41,3] = 4.70$, $P < 0.007$). The post-hoc tests revealed differences between CT 13 and CT 4 ($P < 0.025$), and CT 13 and CT 8 ($P < 0.031$).

time constants of 177 ± 12 ms for CT 13 vs. 206 ± 16 ms for CT 8 (*t*-test, $P = 0.12$). This may indicate that Ca^{2+}-induced Ca^{2+}-channel inactivation is probably not involved in the CT modulation of Ca^{2+}-current amplitude. We then examined whether daytime changes in Ca^{2+} could affect Ca^{2+}-dependent excitability and AHPs, in slices taken at CT 4 and during the early dark period, at CT 13. The resting membrane characteristics (R_N, τ_0, and V_M), firing threshold, and spike width or spike amplitudes, did not differ between the groups (data not shown). A depolarizing pulse induced a train of spikes (> 5) followed by two kinetically distinct AHPs (Fig. 2A). All cells showed a slow AHP (sAHP) and in about 50% of the cells an AHP with kinetic properties resembling the medium AHP (mAHP) [19] preceded the sAHP. Group comparisons revealed neither a change in the peak amplitude of the sAHP, nor of the sAHP amplitude at 3 s after current onset (data not shown). However, the mAHP was significantly reduced at CT 13 compared with CT 4 (−4.0 ± 1.0 mV vs. −9.0 ± 1.0 mV, $P < 0.02$, $n = 6$, Fig. 2A). The after depolarization (ADP) of the spike-repolarization was significantly greater in amplitude in neurons from CT 13 (0.7 ± 0.5, vs. −2.4 ± 0.6 mV, $P < 0.001$, Fig. 2C). Consistent with the decreased mAHP [19], the rate of spike frequency accommodation [5] was significantly reduced by 146 ± 16% in neurons from CT 13 ($P < 0.03$, Fig. 2B). We further studied the extent to which circulating corticosterone levels were involved in the observed diurnal modulation of CA3 excitability. As expected, the highest levels of corticosterone occurred just after the onset of the dark phase (CT 13: 122 ± 10.4 ng/ml; $n = 10$), and were low at CT 23 (21.9 ± 7.2 ng/ml; $n = 4$). Comparison of the averaged Ca^{2+} currents and the individual corticosterone concentrations revealed a U-shaped relationship (Fig. 3A). We also tested the in vitro modulation of CA3 neurons by corticosterone. Slices were taken at CT 4, and 50 nM

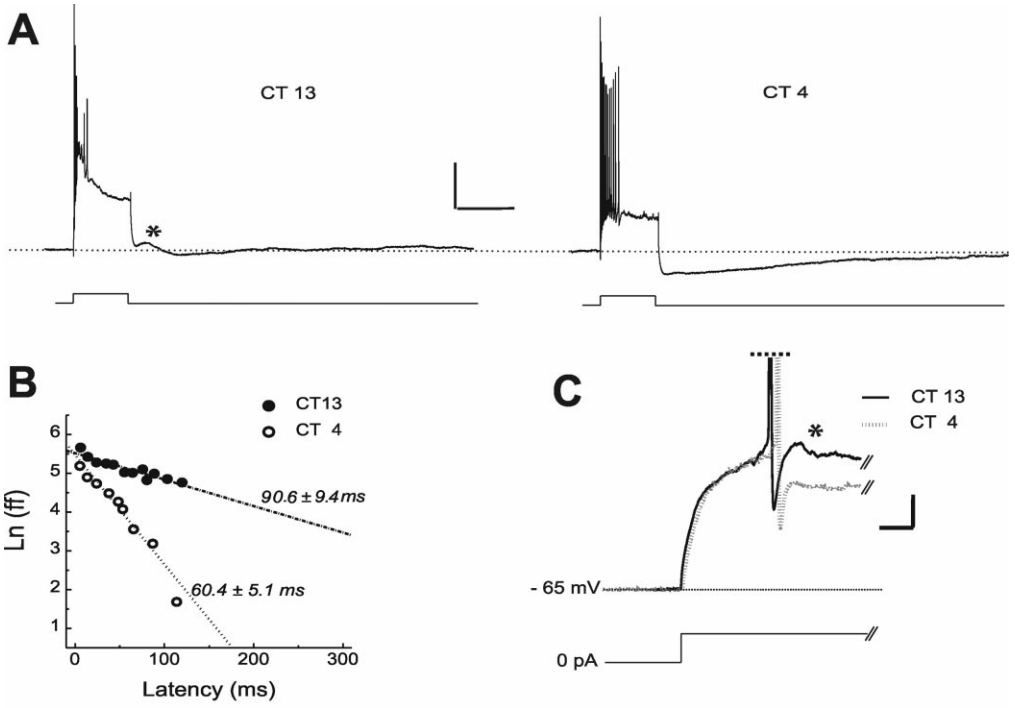

Fig. 2. (A) Current-clamp analysis of CA3 pyramidal neurons performed during the dark (CT 13) and light (CT 4) phases revealed differences in afterpotential characteristics. The AHPs were examined with a 500 ms depolarization of 800 pA at −55 mV (scale bar: 20 mV and 0.5 s). The asterisk marks the suppression of the mAHP ($P < 0.02$). (B) Analysis of the spike accommodation frequency, according to Buckmaster et al. [5], revealed a slower adaptation of spiking during CT 13 compared with CT 4 ($P < 0.03$). (C) Expanded and overlaid traces of single spikes (truncated). The depolarization phase of the post-spike repolarization (ADP, marked by asterisk) was significantly larger in the group of neurons recorded in the dark phase ($P < 0.001$). Scale bar: 20 ms, 5 mv.

($n = 2$) or 100 nM ($n = 4$) corticosterone-hemisuccinate (Steraloids Inc., Wilton, NH, USA) was added to the ACSF incubation medium. Data collection began 1–4 h after application of the steroid hormone. This time interval allowed the measurement of glucocorticoid-elicited effects on gene expression [8,12]. Whole-cell Ca^{2+} currents from CA3 pyramidal neurons (six cells, two rats) were significantly enhanced in the voltage range of −15 to −5 mV (Fig. 3B). Corticosterone selectively increased sAHP (180 ± 23%), spike width (121 ± 4%), and amplitude (108 ± 2%), and reduced accommodation (196 ± 44%: $n = 8$; $P < 0.05$, data not shown).

The present data support a diurnal modulation of hippocampal neurotransmission [2,4,7,14], and demonstrate a dynamic daytime-dependence in the spike afterpotentials and postsynaptic HVA Ca^{2+} currents of CA3 pyramidal neurons. Depolarization-induced excitability exhibited daytime-dependent alterations, possibly related to changes in HVA Ca^{2+}-amplitudes. During the dark phase, the ADP, predominantly generated by Ca^{2+}-influx through either LVA- or HVA-channels [10], was greater than during the light phase. While the changes in Ca^{2+}-current amplitude probably rely on extrahippocampal regulation, we hypothesize that one possible 'zeitgeber' could be the adrenocortical hormone corticosterone that peaks in rats with the onset of the dark period [8], which is the beginning of the activity phase of the animals. Activation of glucocorticoid receptors in CA1 pyramidal cells is known to enhance gene-mediated transcription of HVA L- and P-type, but not N-type Ca^{2+} channels [12]. Mineralocorticoid receptor activation, on the other hand, results in a suppression of Ca^{2+} currents [8]. Therefore, the level of circulating corticosterone exerts a biphasic effect which may account, in particular, for the modulation of Ca^{2+} currents [8,12], and also for the modulation of synaptic plasticity [6,13]. Consistent with the idea of a biphasic regulation, our data show a U-shaped relationship between the time-of-day-dependent measurements of CA3 neuronal HVA Ca^{2+}-amplitudes and circulating corticosterone concentrations. Interestingly, removal of the adrenal glands, eliminating the main source of corticosterone, reverses the circadian rhythm of hippocampal LTP induction [7]. Voltage recordings during the dark phase showed a suppression of the mAHP but no change in the sAHP. This contrasts with the effects of high levels of corticosterone or putatively elevated L-type Ca^{2+} currents, which both increase the sAHP (this study and Refs. [3,16]). Additional factors involved in daytime modulation of CA3 neuronal excitability may include, for instance, acetylcholine. Acetylcholine is increased in the rat hippocampus during the dark period [11], and suppresses Ca^{2+}-dependent K^+ currents, leading to an inhibition of AHPs and spike accommodation [4,18,19]. Daytime variations in the brain-derived neurotrophic factor (BDNF), expressed at higher

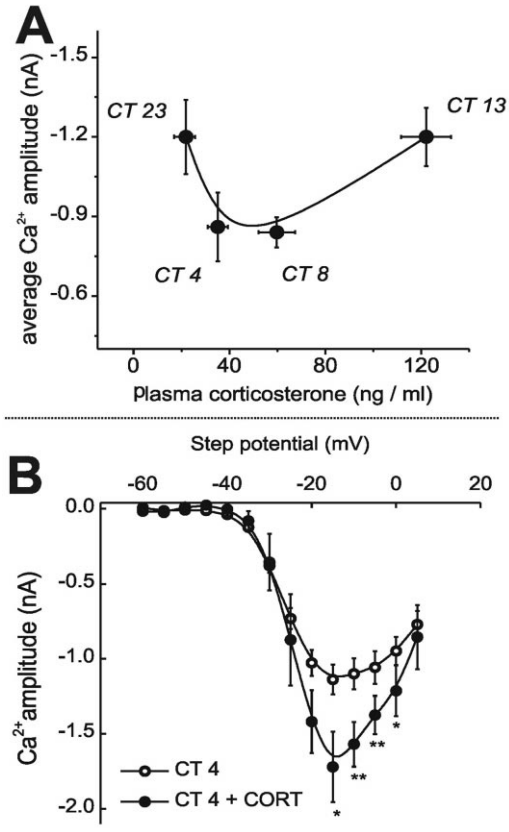

Fig. 3. (A) Individual plasma corticosterone levels plotted against the averaged individual Ca^{2+}-amplitudes revealed a U-shaped relationship between hormone levels and Ca^{2+}-amplitude. (B) Current-voltage relationship of Ca^{2+}-current amplitudes of the CT 4 period compared with current amplitudes recorded after in vitro incubation with 50–100 nM corticosterone. Step potentials at −20, −15, −10, and −5 mV elicited higher amplitudes with corticosterone addition ($*P < 0.05$, $**P < 0.01$).

levels during the dark period [15], may also be involved as BDNF modulates hippocampal synaptic plasticity via HVA Ca^{2+} channels [9].

Our Ca^{2+} current data point to increased intrinsic CA3 neuronal excitability, which is supported by results from Brunel and de Montigny [4], who reported increased CA3 firing and neurotransmitter responsiveness in vivo during the dark phase. Therefore, one may conclude from the present data that the activity phase of the LD-cycle is correlated with different states of intrinsic hippocampal CA3 neuronal properties which are due, to some extent, to altered HVA Ca^{2+} current properties.

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